Microbial Ecology (2022) 84:285–301
https://doi.org/10.1007/s00248-021-01850-4

HOST MICROBE INTERACTIONS

Variations of Phyllosphere and Rhizosphere Microbial Communities of *Pinus koraiensis* Infected by *Bursaphelenchus xylophilus*

Jiaojiao Deng[1] · Dapao Yu[1] · Wangming Zhou[1] · Li Zhou[1] · Wenxu Zhu[2]

Received: 20 April 2021 / Accepted: 23 August 2021 / Published online: 6 September 2021

Abstract

Pine wood nematode, *Bursaphelenchus xylophilus*, as one of the greatest threats to pine trees, is spreading all over the world. Plant microorganisms play an important role in the pathogenesis of nematodes. The phyllosphere and rhizosphere bacterial and fungal communities associated with healthy *Pinus koraiensis* (PKa) and *P. koraiensis* infected by *B. xylophilus* at the early (PKb) and last (PKc) stages were analyzed. Our results demonstrated that pine wood nematode (PWD) could increase the phyllosphere bacterial Pielou_e, Shannon, and Simpson index; phyllosphere fungal Chao 1 index, as well as rhizosphere bacterial Pielou_e, Shannon, and Simpson index; and rhizosphere fungal Pielou_e, Shannon, and Simpson index. What's more, slight shifts of the microbial diversity were observed at the early stage of infection, and the microbial diversity increased significantly as the symptoms of infection worsened. With the infection of *B. xylophilus* in *P. koraiensis*, *Bradyrhizobium* (rhizosphere bacteria), *Massilia* (phyllosphere bacteria), and *Phaeosphaeriaceae* (phyllosphere fungi) were the major contributors to the differences in community compositions among different treatments. With the infection of PWD, most of the bacterial groups tended to be co-excluding rather than co-occurring. These changes would correlate with microbial ability to suppress plant pathogen, enhancing the understanding of disease development and providing guidelines to pave the way for its possible management.

Keywords *Pinus koraiensis* · Pine wood nematode · Phyllosphere microorganism · Rhizosphere microorganism · Network interactions

Introduction

Terrestrial ecosystems are confronted with more and more abiotic and bio-disturbances with the acceleration of global climate change. And extreme climate change aggravates the emergence of plant diseases and insect pests in global forest systems, which has brought great ecological and economic challenges [29]. In the forest ecosystem, the loss of leaves caused by diseases and pests leads to tree dieback and large-scale forest decline, resulting in changes of forest community structure [56], which in turn affects microbial communities [9] and ecosystem function [60]. Plant adaptation to the environment is the result of the integration of the plant itself and its microbiome, which is essential for maintaining the function of terrestrial ecosystems [12, 15, 51, 80]. Over the years, pine trees have been confronted with a severe and devastating disease, pine wilt disease (PWD) mainly caused by *Bursaphelenchus xylophilus* [33], namely the pine wood nematode, which is one of the most serious conifer diseases worldwide, threatening several species of pine trees [78], and resulting in profound economic losses and adverse ecological environmental threat worldwide [67, 78, 85, 95].

Trees infected with PWD have symptoms such as xylem deformation, resin duct disruption, and cortex and cambium tissue damage, which affect water transportation and conduction [26], resulting in the decline in photosynthesis, and ultimately leading to discoloration, wilting, and consequent death of host trees [27]. Given that, the biological characteristics of PWD [50, 101], the dispersing

✉ Li Zhou
zhouli930@iae.ac.cn

✉ Wenxu Zhu
zhuwx@syau.edu.cn

[1] CAS Key Laboratory of Forest Ecology and Management, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

[2] College of Forestry, Shenyang Agricultural University, Shenyang 110866, China

vector [13, 42, 83], and the mechanism of PWD pathogenicity [63, 103] have become research hot spots. In addition, there is growing evidence suggesting that during pathogenesis of *B. xylophilus*, plant microorganisms play important roles in host fitness [31, 34, 74, 81, 84]. The growing amount of data demonstrated that plant-related bacteria have beneficial effects, promote plant growth, and improve plant stress and disease resistance [36, 68], particularly bacterial genera *Trichoderma*, *Serratia*, *Bacillus*, and *Esteya* which have nematicidal activity through mechanisms of parasitism or their production of toxic compounds [53, 89]. In this perspective, there is a pressing need to illuminate the variations of plant microbiome during the disease development [61], advancing our understanding of the relationship between plant compartments and the microbial communities after *B. xylophilus* infection, and paving the way for its possible management.

In recent years, the endophytic microbial community of several *B. xylophilus* host pine trees, such as *Pinus flexilis* [11], *Pinus contorta* [7], *Pinus pinaster* (Proença et al., 2017a), and *Pinus sylvestris* [38], has been well documented. It is well established that pine endophytic bacterial diversity and composition play an important role in regulating plant response to PWN [3, 54, 61]. However, our understanding of the significant implications of phyllosphere and rhizosphere microorganisms remains limited. To the best of our knowledge, phyllosphere and rhizosphere microorganisms are two important components of plant microflora [39]. Previous studies have shown that the decline in plant healthy status or changes in growth conditions caused by host pathogens could affect the microbial community in leaves and roots of the host [23, 76, 85]. The phyllosphere microbiome interacts with the host plant affecting its health and function, and act as mutualists promoting plant growth and tolerance of environmental stressors [72]. The plant healthy status also can affect the exudates of the plant root, which is the essential factor affecting the rhizosphere microbial community [37, 96]. The rhizosphere microbial community has an excellent ability to synthesize diverse secondary metabolites in response to different abiotic and biotic stresses, which is fundamental to the healthy growth of plants [6, 54]. In addition, microorganisms also form a complex and diverse co-occurring network through direct or indirect interactions, and under the action of interspecies relationships such as symbiosis and competition, they produce positive and negative directions between each other, which are closely related to plant resistance [47]. In addition, previous researches collected the samples were only at one time point at the last stage of the disease [52]. Therefore, it is necessary to further investigate the microbial community at different stages of PWD after PWD occurrence and elucidate the relationship between the plant pathogen and host microbial community.

P. koraiensis is widely distributed in Northeast China (Liaoning, Jilin, and Heilongjiang provinces), Japan, North Korea, South Korea, Russia, and other countries, with a total area of about 300,000 km^2 [59]. *P. koraiensis*, as a famous and valuable economic tree species, plays a momentous ecological environmental value. However, in *P. koraiensis*, a main host of *B. xylophilus* that is generally distributed in China, the plant microbiome information after PWD infection under field conditions has been scarcely studied. Here, the phyllosphere and rhizosphere bacterial and fungal communities in healthy *P. koraiensis* (PKa) and *P. koraiensis* naturally infected by *B. xylophilus* at the early stage (PKb) and at the last stage (PKc) of the disease were analyzed by sequencing 16S rDNA and ITS (internal transcribed spacer) rDNA using the high-throughput Illumina NovaSeq PE250 to uncover the differences in host microbial community potentially caused by PWD. In this study, we hypothesized that [2] PWD could increase the diversity of phyllosphere and rhizosphere microbial communities that differed as the infection of *B. xylophilus* progressed; [3] the host bacterial and fungal community differed as the infection of *B. xylophilus* progressed; and [4] with the infection of PWD, most of the microbial taxa tended to be co-excluding; besides, some microbial species unsuited towards living in infected pines disappeared and some species would present. This present study clarified the shifts of the host microbial community of *P. koraiensis* caused by PWD for the first time, so as to better understand the relationships between pathogens and the host microbial community at different stages of PWD after the occurrence of PWD. What's more, microbial community composition and microbial network can be used as biological indicators at different stages after the occurrence of PWD.

Materials and Methods

Overview of the Research Area

The study area is located at Dengta City, Liaoyang City, Liaoning Province, China (41°17′44″ N, 123°35′47″ E). The climate in this area is characterized as north temperate continental climate with an annual average temperature of 8.8 °C, annual average precipitation of 600 to 800 mm, and an annual average frost-free period of 140 to 160 days. The soil type is classified as Eutrochrepts soil [71]. Five fixed sites with the same site conditions and soil basic properties were selected, with an area of 1 ha. Eight healthy *P. koraiensis* trees (PKa), eight diseased *P. koraiensis* trees infected by *B. xylophilus* at the early stage (PKb), and eight diseased *P. koraiensis* trees at the last stage (PKc) were selected for sampling in each site. The distance between diseased and adjacent healthy trees was less than 15 m. The selection of healthy and *P. koraiensis* trees infected by *B. xylophilus*

was made in accordance with the method described by Millberg et al. [57], in which the healthy trees were completely green needles and from which no *B. xylophilus* was isolated. The early stage of infection tree refers to needles that have become slightly wilted and browning of needles. The last stage of diseased trees was completely dead looking with brown needles (Fig. S1).

Sample Collection

For the leaf sampling, 24 needle samples were collected from the tops of the branches from three directions (120° as the boundary) from 8 trees, and mixed as one replicate at each site. A total of 15 leaf samples (3 types × 5 replicates) were collected from the study area. With regard to soil sampling, 24 rhizosphere soils were collected from three directions from 8 trees, and pooled together as one replication in each site, resulting in a total of 15 rhizosphere soil samples (3 types × 5 replicates). All the samples were put in the icebox and transported to the laboratory for subsequent analysis.

To analyze the microbial community on the leaves according to Kembel and Mueller [43], and Ren et al. [65], 10 g of leaf samples from each replicate was cut into pieces and transferred to a sterile triangle flask. 1:20 (leaf weight/volume TE buffer =1:20) phosphate-buffered saline solution (20 ml, PBS, 0.01 M, pH 7.4) was added to each triangle flask. After sealed with a sterilized film, the samples were shaken on a shaker at 200 r/min for 30 min at room temperature, and the microbial cells were separated from the leaf surface. Vacuum filtration was used in a sterile environment, and microbes from the oscillating liquid were collected on a 0.22-μm microporous membrane placed into 2-ml sterile centrifuge tubes, then stored at −80 °C prior to DNA extraction. Fresh soil removed plant residues and stone was passed through a 2-mm sieve and immediately put into 2-ml sterile centrifuge tubes and frozen at −80 °C for later DNA extraction and high-throughput sequencing.

DNA Extraction, Amplification, and NovaSeq PE250 Sequencing

Genomic DNA was extracted from microporous membranes and 0.5 g soil using the FastDNA SPIN Kit for soil (MP Biomedicals, Santa Ana, CA, USA), in accordance with the manufacturer's instructions. The concentrations of DNA were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). The V3-V4 regions of the bacterial 16S rDNA gene were amplified and sequenced using the primer pairs 338F (5′-ACTCCTACG GGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGG TWTCTAAT-3′) with barcode sequence. And ITS1 regions of the fungal ITS rDNA gene were amplified using the primer pairs ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) with barcode sequence [20]. All the PCR were carried out with 25-μl mixture, including 2 μl of dNTPs (2.5 mM); 2 μl DNA template (40–50 ng); 0.25 μl (5 U/μl) of Q5 High-Fidelity DNA Polymerase; 8.75 μl of ddH_2O; 5 μl of Q5 High-Fidelity GC buffer (5×) and Q5 reaction buffer (5×), respectively, 1 μl (10 uM) of forward primer; and 1 μl (10 uM) of reverse primer. The following PCR thermal cycling conditions consisted of an initial denaturation step for 5 min at 98 °C, followed by 25 cycles of denaturation for 15 s at 98 °C, annealing for 30 s at 55 °C, and elongation of 72 °C for 30 s, with the final elongation step for 5 min at 72 °C. The PCR amplicons were further purified and quantified by using Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) and PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). PCR products for sequencing were carried out using an Illumina's NovaSeq PE250 platform at Shanghai Personal Biotechnology Co., Ltd., Shanghai, China. The high-throughput sequencing raw data of phyllosphere and rhizosphere microbes were uploaded in the NCBI database with the SRA accession numbers of PRJNA689361 and PRJNA689392.

Bioinformation Analysis

After removing primers and barcode sequences with cutadapt, quality filter, denoise, joint, and removal of chimeras, the high-quality sequences were finally obtained. Sequences with ≥97% similarity were assigned to the same OTU. The Silva Database for bacteria (Release132, http://www.arb-silva.de) [64] and Unite Database for fungi (Release 8.0, https://unite.ut.ee/) [46] were used for each representative sequence.

Statistical Analysis

One-way analysis of variance (ANOVA) with an LSD test was used to identify differences in microbial community richness (Chao 1 index, Observed species), diversity (Shannon index, Simpson index), and evenness (Pielou_e index) among different treatments. Venn diagrams were constructed using subsampled data to show the shared and unique OTUs in RStudio with the "Venn" package. Linear discriminant analysis Effect Size (LEfSe) in Galaxy software was employed to identify microbial lineages (from the phylum to genus) responsible for the differentiation of the phyllosphere and rhizosphere microbial communities caused by different treatments. Principal coordinate analysis (PCoA) based on the Bray-Curtis distance matrix, as one of the classical multidimensional scaling, was used to visualize the distinction of the microbial community structure. Heatmap plots of phyllosphere and rhizosphere microbial communities with

the relative abundance of top 50 at the genus level were performed based on the Bray-Curtis distance matrix using RStudio with the package "Vegan". The co-occurrence patterns of OTUs from different treatments were evaluated by network analysis using the "psych" package in RStudio based on the Spearman rank correlation, and Gephi software was applied to visualize the networks with a Fruchterman-Reingold layout.

Results

Changes in Phyllosphere and Rhizosphere Microbial Community Alpha Diversity

A total of 1,367,262 and 1,174,114 high-quality phyllosphere and rhizosphere bacterial sequences were generated across all samples after sequence denoising and quality filtering with the average number of sequences per sample 91,150 and 78,274, severally, which were assigned into 11,294 and 18,175 OTUs. The number of shared phyllosphere bacterial OTUs among PPKa, PPKb, and PPKc was 1306, and the unique OTUs of PPKa, PPKb, and PPKc were 2400, 3028, and 2862, respectively (Fig. 1A). The number of shared rhizosphere bacterial OTUs among RPKa, RPKb, and RPKc was 2297, and the unique OTUs of RPKa, RPKb, and RPKc were 3416, 3145, and 5437, respectively (Fig. 1B). Moreover, the shared OTUs among RPKa, RPKb, RPKc, PPKa, PPKb, and PPKc were 13 (Fig. S2A).

The fungal communities were further explored by high-throughput amplicon sequencing. Across all samples, we obtained a total of 1,318,977 and 1,316,090 high-quality phyllosphere and rhizosphere fungal sequences after sequence denoising and quality filtering with the average number of sequences per sample 87,739 and 87,931, severally, which were respectively grouped into 1272 and 3190 OTUs. The number of shared phyllosphere fungal OTUs among PPKa, PPKb, and PPKc was 276, and the number of unique OTUs of PPKa, PPKb, and PPKc was 599, 846, and 997, respectively (Fig. 1C). The number of shared rhizosphere fungal OTUs among RPKa, RPKb, and RPKc was 269, and the number of unique OTUs of RPKa, RPKb, and RPKc was 272, 260, and 245, severally (Fig. 1D). In addition, the shared OTUs among RPKa, RPKb, RPKc, PPKa, PPKb, and PPKc were 23 (Fig. S2B).

As expected, there was considerable variation of phyllosphere bacterial Pielou_e ($F = 12.639$, $P = 0.001$), Shannon ($F = 10.268$, $P = 0.003$), and Simpson index ($F = 5.882$, $P = 0.017$) among PPKa, PPKb, and PPKc. Furthermore,

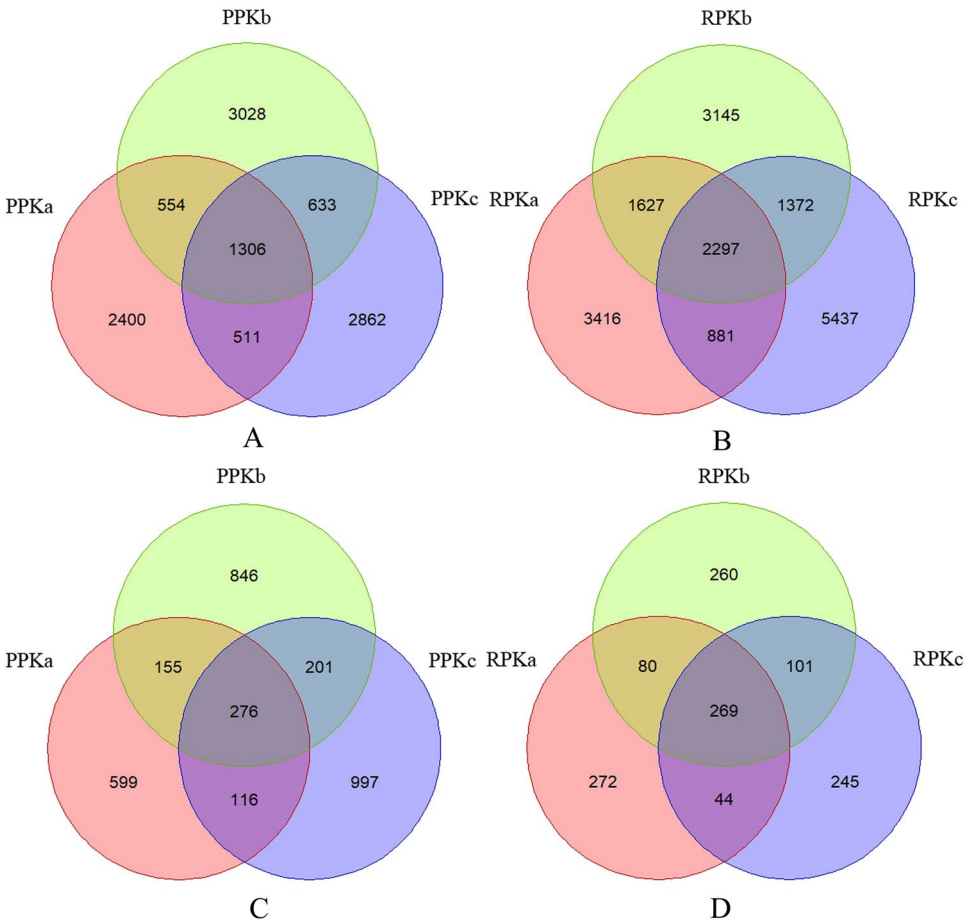


Fig. 1 The Venn diagrams of phyllosphere bacterial OTUs (**A**), rhizosphere bacterial OTUs (**B**), phyllosphere fungal OTUs (**C**), and rhizosphere fungal OTUs (**D**). PPKa, the phyllosphere of healthy *Pinus koraiensis*; PPKb, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; PPKc, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage; RPKa, the rhizosphere of healthy *P. koraiensis*; RPKb, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; RPKc, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage

relative to PPKa and PPKb, PPKc increased phyllosphere bacterial Pielou_e, Shannon, and Simpson index with 0.72, 7.83, and 0.97, respectively (Table 1). In addition to Goods_coverage ($F=3.533$, $P=0.062$) and Simpson index ($F=3.235$, $P=0.075$), rhizosphere bacterial Observed_species ($F=6.777$, $P=0.011$), Chao 1 ($F=4.655$, $P=0.032$), Pielou_e ($F=47.496$, $P=0.0001$), and Shannon index ($F=11.772$, $P=0.002$) were observed with significant differences among RPKa, RPKb, and RPKc. What's more, RPKc holds the highest rhizosphere bacterial Observed_species, Chao 1, Pielou_e, Shannon, and Simpson index with 5384.50, 6916.43, 0.895, 11.09, and 0.9987, separately (Table 2). With regard to fungi, phyllosphere fungal Chao 1 index ($F=56.306$, $P=0.000$), Goods_coverage ($F=14.509$, $P=0.001$), and Observed_species ($F=56.689$, $P=0.000$) differed dramatically among PPKa, PPKb, and PPKc, and PPKc holds the highest Chao 1 index with 597.46 (Table 1). Rhizosphere fungal Pielou_e ($F=12.639$, $P=0.001$), Shannon ($F=10.268$, $P=0.003$), and Simpson index ($F=5.882$, $P=0.017$) among RPKa, RPKb, and RPKc also appeared obviously different. It is well established that RPKc owned highest rhizosphere fungal Pielou_e, Shannon, and Simpson index with 0.56, 4.66, and 0.89 (Table 2).

Table. 1 Phyllosphere microbial community diversity among PPKa, PPKb, and PPKc. *PPKa*, the phyllosphere of healthy *Pinus koraiensis*; *PPKb*, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; *PPKc*, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage. Mean ± standard error, $n=5$. Different lowercase letters in the same row indicate significant differences at the 0.05 level

Phyllosphere bacterial community diversity	PPKa	PPKb	PPKc	*F*	*P*
Chao 1 index	2050.54 ± 169.79 a	2171.61 ± 272.80 a	2508.21 ± 142.48 a	1.366	0.292
Goods_coverage	0.985 ± 0.001 a	0.985 ± 0.003 a	0.983 ± 0.001a	0.584	0.573
Observed_species	1533.26 ± 126.09 a	1689.48 ± 201.14 a	1927.50 ± 106.01 a	1.749	0.215
Pielou_e index	0.61 ± 0.02 b	0.64 ± 0.01 b	0.72 ± 0.01 a	12.639	0.001
Shannon index	6.40 ± 0.30 b	6.85 ± 0.19 b	7.83 ± 0.19 a	10.268	0.003
Simpson index	0.93 ± 0.01 b	0.95 ± 0.01 ab	0.97 ± 0.00 a	5.882	0.017
Phyllosphere fungal community diversity	PPKa	PPKb	PPKc	*F*	*P*
Chao 1 index	451.26 ± 19.57 c	548.89 ± 20.17 b	597.46 ± 26.21 a	56.306	0.000
Goods_coverage	0.9994 ± 0.0002 a	0.9994 ± 0.0001 a	0.9991 ± 0.000 b	14.509	0.001
Observed_species	441.64 ± 17.76 c	538.74 ± 19.80 b	582.20 ± 25.75 a	56.689	0.000
Pielou_e index	0.653 ± 0.035 a	0.637 ± 0.010 a	0.635 ± 0.009 a	0.976	0.405
Shannon index	5.74 ± 0.33 a	5.78 ± 0.12 a	5.84 ± 0.11 a	0.277	0.763
Simpson index	0.96 ± 0.02 a	0.94 ± 0.01 b	0.95 ± 0.00 ab	3.321	0.071

Variations in Phyllosphere and Rhizosphere Microbial Community Beta Diversity

It is well established that the microbial compositions from rhizosphere and phyllosphere samples formed distinct clusters (Fig. 2), which indicated that the plant compartment is a major selective force for the formation of plant-related microbial composition. The unconstrained principal coordinate analysis (PCoA) of the Bray-Curtis distance from all phyllosphere and rhizosphere samples based on the OTU data detected 64.9% of the total variance among bacterial communities, with the first and second axes explaining 57.2% and 7.7% of the variance, respectively (Fig. 2A). PCoA based on the OTU data detected 67.0% of the total variance of fungal communities, with the first and second axes explaining 55.7% and 11.3% of the variance, respectively (Fig. 2B). As expected, the infection of *B. xylophilus* had a profound effect on plant microbe. The results demonstrated that rhizosphere bacterial community (Fig. S3A), rhizosphere fungal community (Fig. S3C), and phyllosphere fungal community (Fig. S3D) from PKa, PKb, and PKc formed three distinct clusters, especially along the PCoA1.

Comparative Analysis of Phyllosphere and Rhizosphere Microbial Community Composition

For bacteria, at the phylum level, 36 rhizosphere bacterial groups were obtained, and 8 bacterial communities with the relative abundance more than 1% were detected, including Proteobacteria, Actinobacteria, Acidobacteria, Verrucomicrobia, Chloroflexi, Gemmatimonadetes, Patescibacteria, Bacteroidetes, and Firmicutes, accounting for 94.75% (Fig. 3A). In total, 27 phyllosphere bacterial groups were obtained, and 4 bacterial communities with the relative abundance more than 1% were obtained, including Proteobacteria, Actinobacteria, Bacteroidetes, and Cyanobacteria (Fig. 3B). As for fungi, at the phylum level, 7 rhizosphere fungal groups were obtained, and 2 fungal communities with the relative abundance more than 1% were detected, including Ascomycota and Basidiomycota (Fig. 4A). Fourteen phyllosphere fungal groups were obtained, and 4 fungal

Table 2 Rhizosphere microbial community diversity among RPKa, RPKb, and RPKc. *RPKa*, the rhizosphere of healthy *Pinus koraiensis*; *RPKb*, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; *RPKc*, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage. Mean ± standard error, $n = 5$. Different lowercase letters in the same row indicate significant differences at the 0.05 level

Rhizosphere bacterial community diversity	RPKa	RPKb	RPKc	*F*	*P*
Chao 1 index	5653.84 ± 174.84 b	5604.88 ± 553.16 b	6916.43 ± 140.37 a	4.655	0.032
Goods_coverage	0.961 ± 0.002 a	0.962 ± 0.006 a	0.951 ± 0.002 b	3.533	0.062
Observed_species	4434.48 ± 112.81 b	4445.78 ± 332.54 b	5384.50 ± 91.15 a	6.777	0.011
Pielou_e index	0.883 ± 0.001 c	0.886 ± 0.001 b	0.895 ± 0.001 a	47.496	0.000
Shannon index	10.70 ± 0.04 b	10.72 ± 0.10 b	11.09 ± 0.02 a	11.772	0.002
Simpson index	0.99846 ± 0.0001 b	0.99852 ± 0.0001 ab	0.9987 ± 0.0000 a	3.235	0.075
Rhizosphere fungal community diversity	RPKa	RPKb	RPKc	*F*	*P*
Chao 1 index	287.32 ± 24.81 a	311.38 ± 20.81 a	325.84 ± 11.91 a	0.954	0.412
Goods_coverage	0.9996 ± 0.0000 a	0.9995 ± 0.0001 a	0.9995 ± 0.0001 a	0.346	0.714
Observed_species	279.30 ± 24.08 a	300.10 ± 18.09 a	315.14 ± 10.71 a	0.951	0.414
Pielou_e index	0.45 ± 0.02 b	0.47 ± 0.03 b	0.56 ± 0.01 a	8.837	0.004
Shannon index	3.63 ± 0.20 b	3.85 ± 0.26 b	4.66 ± 0.10 a	7.825	0.007
Simpson index	0.76 ± 0.03 b	0.81 ± 0.03 ab	0.89 ± 0.01 a	5.889	0.017

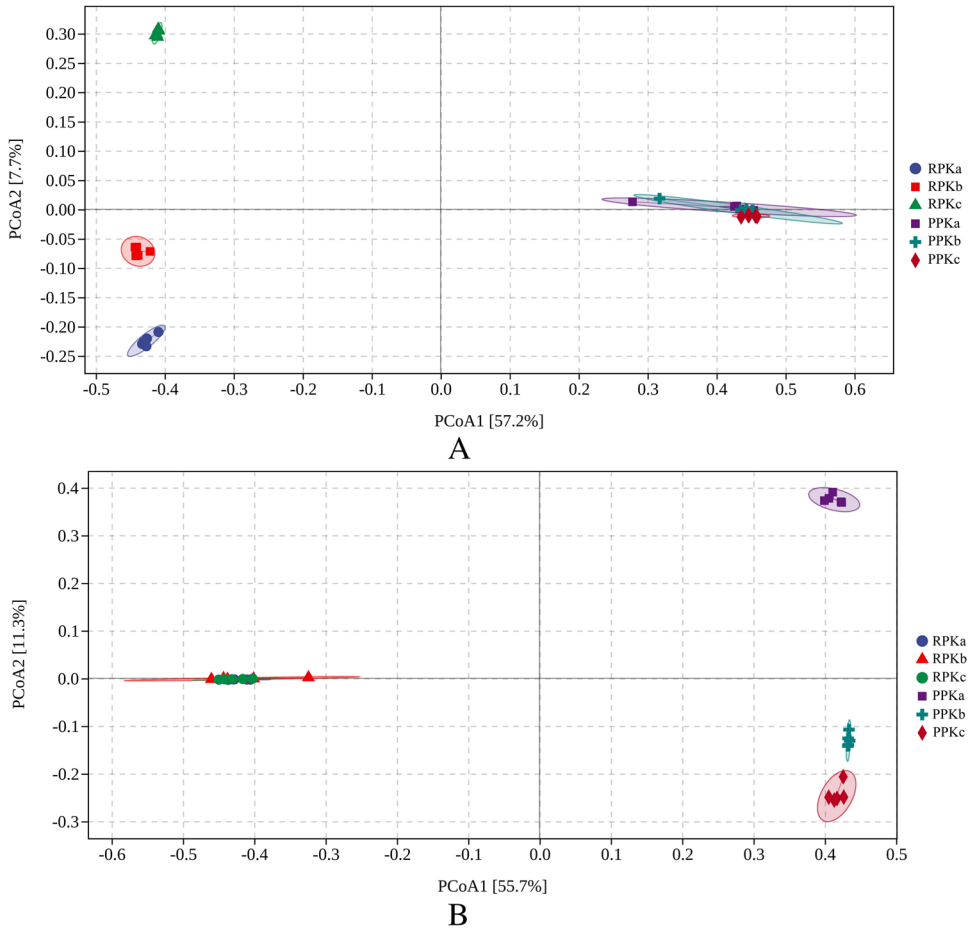


Fig. 2 PCoA (principal coordinate analysis) based on the Bray-Curtis distance of bacterial (**A**) and fungal (**B**) communities from phyllosphere and rhizosphere among different samples. PPKa, the phyllosphere of healthy *Pinus koraiensis*; PPKb, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; PPKc, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage; RPKa, the rhizosphere of healthy *P. koraiensis*; RPKb, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; RPKc, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage

communities with the relative abundance more than 1% were detected, including Basidiomycota, Ascomycota, Mortierellomycota, and Mucoromycota (Fig. 4B).

At the genus level, 851 rhizosphere bacterial communities were obtained, of which, the average relative abundance of *Candidatus_Udaeobacter*, *Mycobacterium*, *Acidothermus*, *AD3*, *Subgroup_6*, *KD4–96*, *Saccharimonadales*, *Subgroup_2*, *Bradyrhizobium*, *Pseudolabrys*, *Ellin6067*, *Burkholderia-Caballeronia-Paraburkholderia*, *Gaiella*, *Bryobacter*, *IMCC26256*, and *67–14* was more than 1% (Fig. S4A). In total, 606 phyllosphere bacterial communities were obtained, and the relative abundance of *Methylobacterium*,

Pantoea, *Sphingomonas*, *1174–901-12*, *Hymenobacter*, *Amnibacterium*, *Massilia*, *Pseudomonas*, *Chloroplast*, *Enterobacter*, *P3OB-42*, *Rosenbergiella*, and *Endobacter* was more than 1% (Fig. S4B). Heatmap demonstrated that rhizosphere (Fig. 5A) and phyllosphere (Fig. 5B) bacteria from RPKa (PPKa) and RPKb (PPKb) formed a cluster, clearly distinguished from those of RPKc (PPKc). For fungi, 321 rhizosphere fungal communities were obtained, among which, the groups with the relative abundance more than 1% were *Didymella*, *Alternaria*, *Selenophoma*, *Septoria*, *Aureobasidium*, *Genolevuria*, *Phialemoniopsis*, and *Taphrina* (Fig. S4C). In total, 492 phyllosphere fungal communities were obtained, and the groups with the relative abundance more than 1% were *Mortierella*, *Russula*, *Sebacina*, *Saitozyma*, *Suillus*, *Phialocephala*, *Chalara*, *Trechispora*, *Ilyonectria*, *Solicoccozyma*, *Trichocladium*, *Amphinema*, *Penicillium*, *Fusarium*, *Umbelopsis*, *Tomentella*, and *Exophiala* (Fig. S4D). Heatmap demonstrated that rhizosphere (Fig. 5C) and phyllosphere (Fig. 5D) fungi from RPKb (PPKb) and RPKc (PPKc) formed a cluster, and clearly distinguished from those of RPKa (PPKa).

Furthermore, we conducted LEfSe analysis to identify which microbial taxa (from phylum to genus level) were major contributors to the differences in rhizosphere and phyllosphere community compositions among different samples (Fig. 6). At the phylum level, the larger groups of rhizosphere bacteria in RPKa were Actinobacteria, Gemmatimonadetes, and Patescibacteria, while in RPKb were Chloroflexi, Rokubacteria, and Verrucomicrobia, and in RPKc were Acidobacteria, Bacteroidetes, and Proteobacteria (Kruskal-Wallis test, $P < 0.05$) (Fig. 6A). The larger

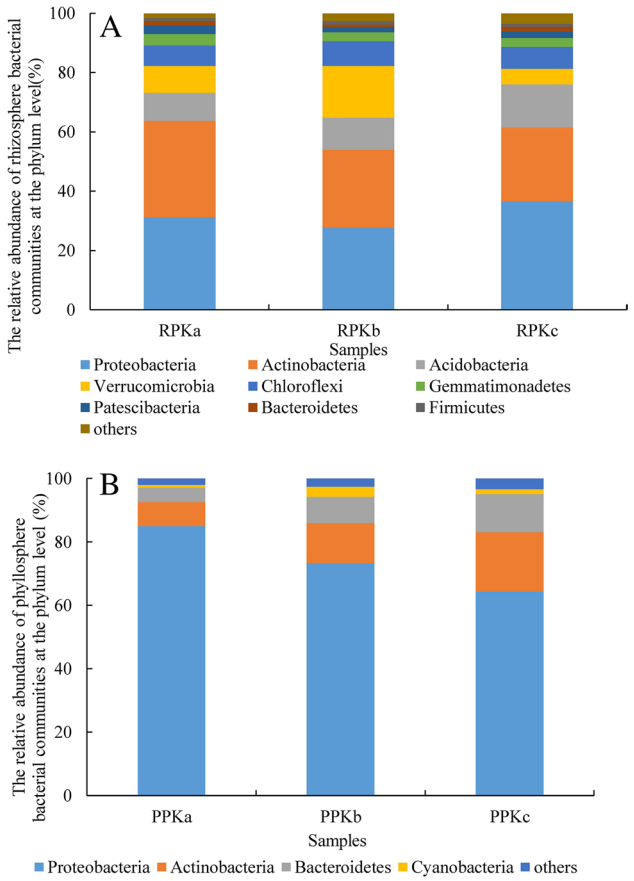


Fig. 3 The relative abundance of rhizosphere (**A**) and phyllosphere (**B**) bacterial communities at the phylum level among different samples. PPKa, the phyllosphere of healthy *Pinus koraiensis*; PPKb, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; PPKc, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage; RPKa, the rhizosphere of healthy *P. koraiensis*; RPKb, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; RPKc, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage

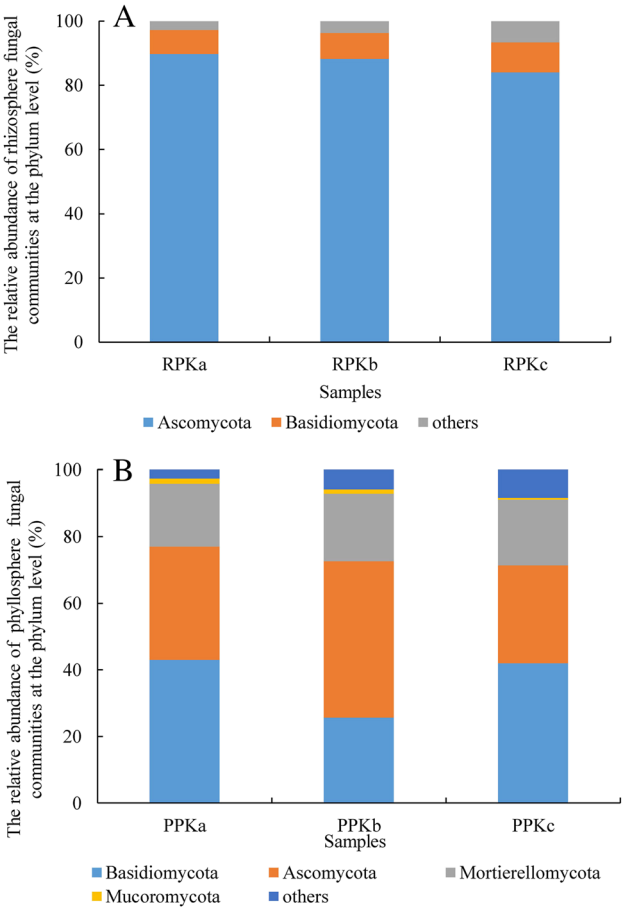


Fig. 4 The relative abundance of rhizosphere (**A**) and phyllosphere (**B**) fungal communities at the phylum level among different samples. PPKa, the phyllosphere of healthy *Pinus koraiensis*; PPKb, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; PPKc, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage; RPKa, the rhizosphere of healthy *P. koraiensis*; RPKb, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; RPKc, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage

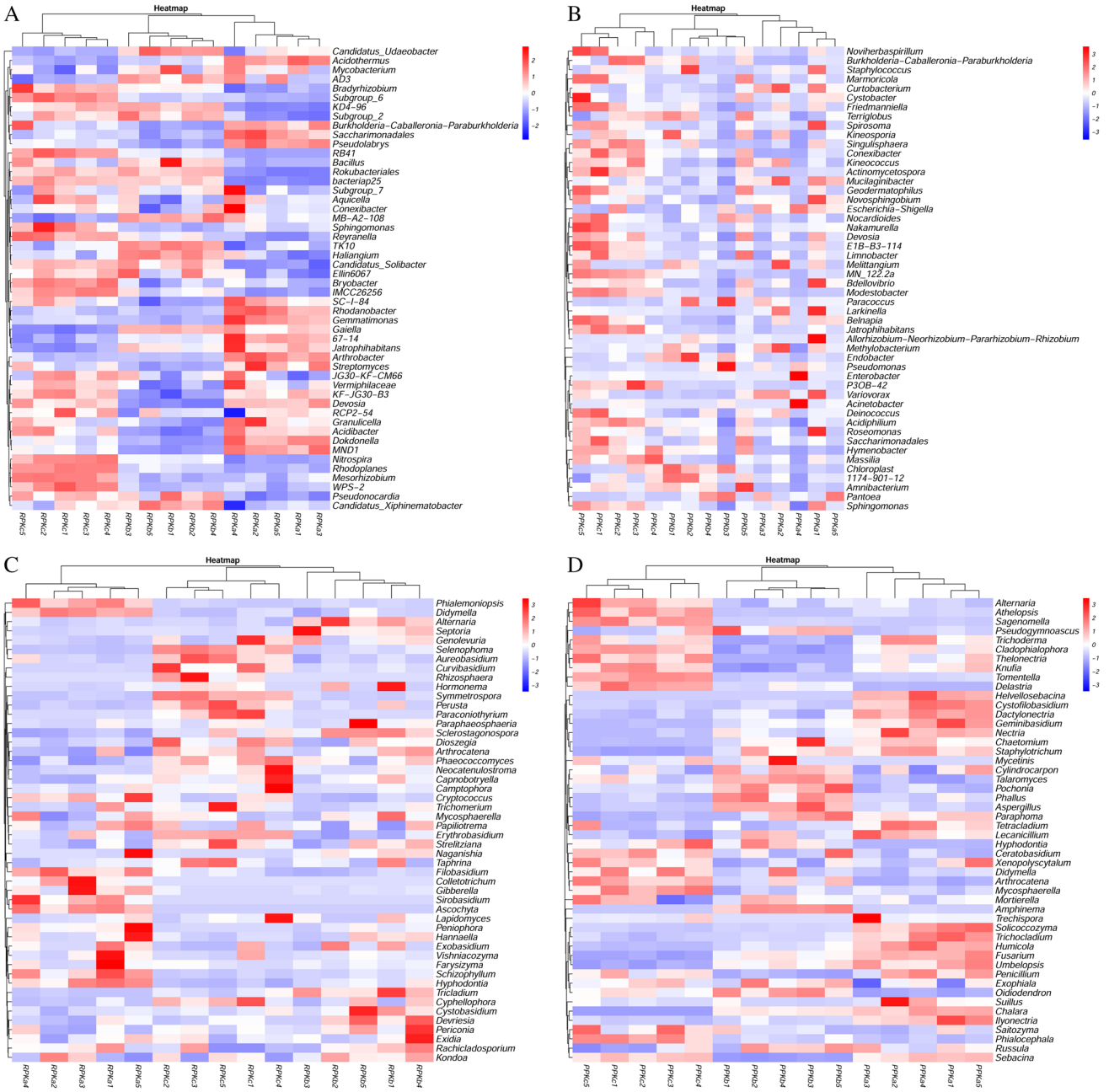


Fig. 5 Heatmap of rhizosphere bacterial (**A**), phyllosphere bacterial (**B**), rhizosphere fungal (**C**), and phyllosphere fungal (**D**) communities with the relative abundance at the top 50. PPKa, the phyllosphere of healthy *Pinus koraiensis*; PPKb, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; PPKc, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage; RPKa, the rhizosphere of healthy *P. koraiensis*; RPKb, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; RPKc, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage

group of phyllosphere bacterial group in PPKa was P roteobacteria, while in PPKb were Acidobacteria, and Chloroflexi, and in PPKc were Actinobacteria, Armatimonadetes, Bacteroidetes, and Planctomycetes ($P < 0.05$) (Fig. 6B). Additionally, the rhizosphere fungi groups of Chalara, Basidiomycota, Mucoromycota, and Staphylotrichum were significantly enriched in RPKa, while Rozellomycota, Basidiomycota and Arthrocatena were more enriched in RPKc as compared to RPKa and RPKb ($P < 0.05$). The phylum Ascomycota was more abundant in RPKb than RPKa and RPKc (Fig. 6C). For phyllosphere fungi, the PPKa contained a significantly higher abundance of Phialemoniopsis

than PPKb and PPKc samples ($P < 0.05$), while PPKb owned higher abundances of Ascomycota and Pseudovirgaria. Curvibasidium, Neophaeococcomyces, Selenophoma, and Symmetrosporaceae presented higher in RPKc (Fig. 6D).

Microbiological Information Network and co-Occurrence Analysis

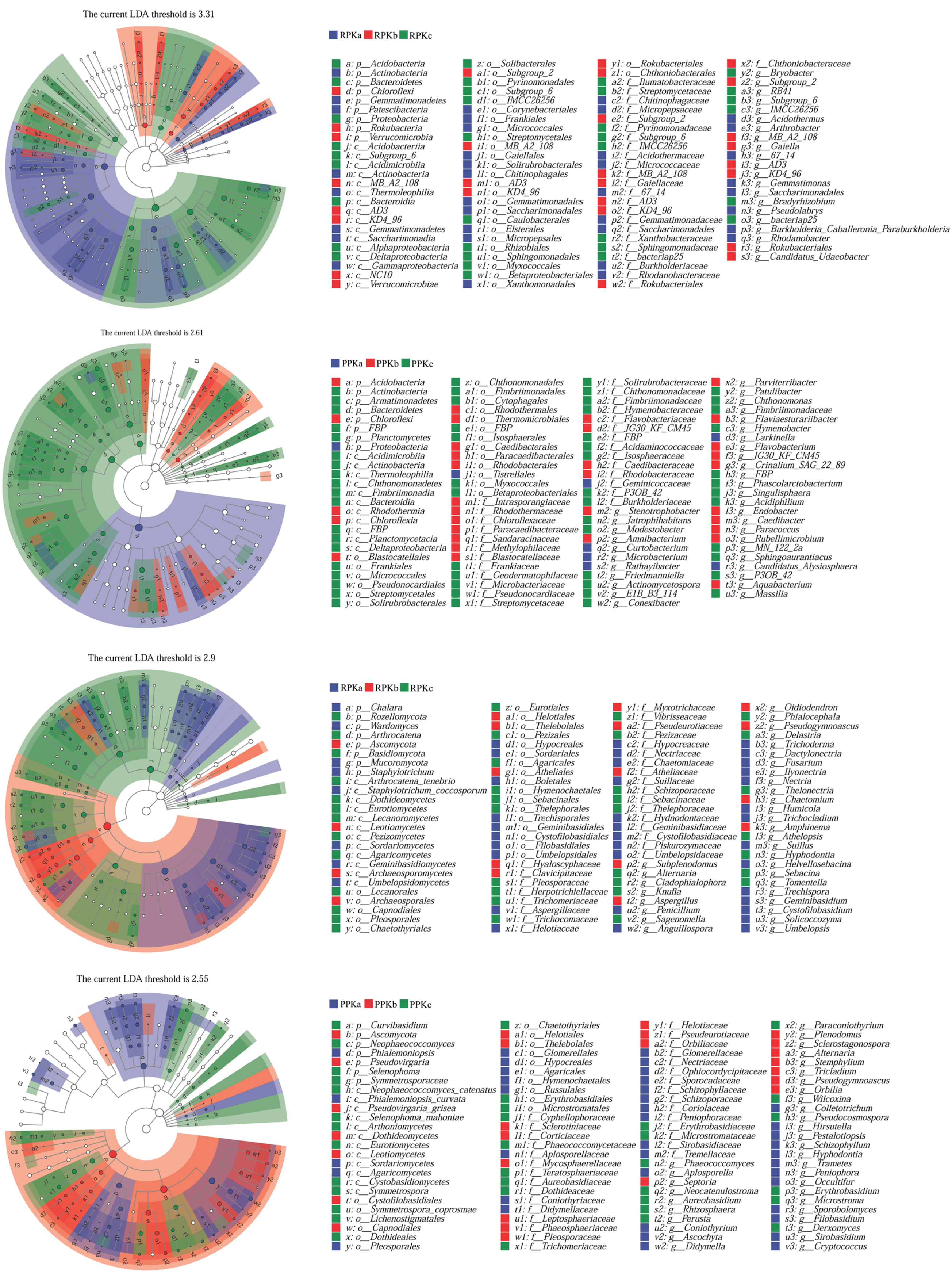
In order to further disentangle complex microbe-microbe interactions, we created association networks of phyllosphere and rhizosphere bacterial and fungal communities from OTU data (Fig. 7; Table S1). Total nodes of phyllosphere and rhizosphere bacterial community association network in PKc existed the highest, followed by PKb and PKa, and total nodes of phyllosphere and rhizosphere fungal community association network in PKb existed the highest, followed by PKc and PKa, both indicating that the OTUs of the ecological network increased after infection (Fig. 7; Table S1). Graph density in the network of PKb, a key topological property to describe how well a node is connected with its neighbors, showed higher than PKa and PKc, suggestive of more intensive microbial coupling at the early stage of infection (Fig. 7; Table S1). Except for phyllosphere fungi, positive links showed decreased with the infection of PWD, and at the last stage, positive links existed the lowest (Fig. 7; Table S2), demonstrating that most of the microbial taxa tended to be co-excluding rather than co-occurring.

Discussion

Microbial Community Diversity Response to Different Samples

Plant microorganisms play critical roles in ecosystem function, sustainable restoration and management, as well as health of many plant species ([18, 45, 58, 61, 77]. As climate change and human activity disrupt natural environments and microbial processes, there is essential to further explore the variations of microbe-microbe interactions [30] and microbe-host interactions [17]. We investigated the microbial community of phyllosphere and rhizosphere from healthy and diseased pine trees naturally infected by *B. xylophilus* at the different stages under field conditions. In our study, 11,294 and 18,175 phyllosphere and rhizosphere bacterial OTUs, and 1272 and 3190 phyllosphere and rhizosphere fungal OTUs of healthy and diseased pines were detected. In almost all samples, the rhizosphere bacterial Chao 1 index, Pielou_e, Shannon, and Simpson index were much higher than the respective phyllosphere communities (Table 1), which was a common finding in similar studies of native and cultivated plants in different environments [8, 22, 102]. The differences in microbial community diversity between the two plant compartments might account for the direct influence of their surrounding environment, and their fundamental discrimination of physiology and function [28]. Mounting empirical evidences have suggested that root exudates have a strong detrimental role in selecting the growth of specific bacteria [10, 88] through signal transmission of microbe-microbe and plant-microbe interactions [82], ultimately promoting the differentiation of the bacterial assemblages [8]. Additionally, phyllosphere exists generally lower bacterial richness and abundance due to the fluctuations in environmental pressures [79, 87]. In regard to fungal community, phyllosphere fungal community diversity presented higher than rhizosphere fungal community diversity (Table 2), which was not in agreement with previous researches from Chen et al. [14] and Jia et al. [40]. Our results nicely demonstrated that the effects of root and leaf compartments on the α-diversity indices of fungal community were different from those of bacterial community [48, 73].

What's more, phyllosphere and rhizosphere microbial community diversity between healthy and infected *P. koraiensis* presented obvious difference. At the early stage of the infection, rhizosphere and phyllosphere bacterial Pielou_e and Simpson index, rhizosphere fungal Shannon and Simpson index, as well as phyllosphere fungal Chao 1 index exhibited slightly higher than those of healthy *P. koraiensis* (Table 1; Table 2). At the last stage of the infection, rhizosphere and phyllosphere bacterial Pielou_e, Shannon, and Simpson index; rhizosphere fungal Pielou_e, Shannon, and Simpson index; and phyllosphere fungal Chao 1 index existed abundantly higher than those of healthy *P. koraiensis* and the early stage of the infection (Tables 1 and 2). Our findings were consistent with a previous study from Proença et al. [62] who demonstrated that the endophytic bacterial diversity of *P. pinaster* tree was the highest at the late stage of pine wood nematode infection, and there was no conspicuous difference in bacterial diversity at the early stage of the disease, while the research from Ma et al. [52] indicated that there were no significant differences of rhizospheric bacterial diversities between healthy and wilted pines. Besides another investigation that showed that *B. xylophilus* infection appeared to reduce soil bacterial diversity [69], similar findings were reported by Zhang et al. [97] who demonstrated that *B. xylophilus* infection likely decreased the richness and diversity of endophytic microbes. It thus appeared that the inconsistent results might be due to different tree species and the sampling period after the infection of PWD. In the present study, we revealed that PWD could increase the phyllosphere and rhizosphere bacterial and fungal diversity and the microbial community diversity differed as the disease progressed, suggesting the importance of the host microbiome in disease development. The differences might be caused by the growing abundance of the dominant


The current LDA threshold is 3.31
RPKa RPKb RPKc
a: p__Acidobacteria
b: p__Actinobacteria
c: p__Bacteroidetes
d: p__Chloroflexi
e: p__Gemmatimonadetes
f: p__Patescibacteria
g: p__Proteobacteria
h: p__Rokubacteria
i: p__Verrucomicrobia
j: c__Acidobacteriia
k: c__Subgroup_6
l: c__Acidimicrobiia
m: c__Actinobacteria
n: c__MB_A2_108
o: c__Thermoleophilia
p: c__Bacteroidia
q: c__AD3
r: c__KD4_96
s: c__Gemmatimonadetes
t: c__Saccharimonadia
u: c__Alphaproteobacteria
v: c__Deltaproteobacteria
w: c__Gammaproteobacteria
x: c__NC10
y: c__Verrucomicrobiae
z: o__Solibacterales
a1: o__Subgroup_2
b1: o__Pyrinomonadales
c1: o__Subgroup_6
d1: o__IMCC26256
e1: o__Corynebacteriales
f1: o__Frankiales
g1: o__Micrococcales
h1: o__Streptomycetales
i1: o__MB_A2_108
j1: o__Gaiellales
k1: o__Solirubrobacterales
l1: o__Chitinophagales
m1: o__AD3
n1: o__KD4_96
o1: o__Gemmatimonadales
p1: o__Saccharimonadales
q1: o__Caulobacterales
r1: o__Elsterales
s1: o__Micropepsales
t1: o__Rhizobiales
u1: o__Sphingomonadales
v1: o__Myxococcales
w1: o__Betaproteobacteriales
x1: o__Xanthomonadales
y1: o__Rokubacteriales
z1: o__Chthoniobacterales
a2: f__Ilumatobacteraceae
b2: f__Streptomycetaceae
c2: f__Chitinophagaceae
d2: f__Micropepsaceae
e2: f__Subgroup_2
f2: f__Pyrinomonadaceae
g2: f__Subgroup_6
h2: f__IMCC26256
i2: f__Acidothermaceae
j2: f__Micrococcaceae
k2: f__MB_A2_108
l2: f__Gaiellaceae
m2: f__67_14
n2: f__AD3
o2: f__KD4_96
p2: f__Gemmatimonadaceae
q2: f__Saccharimonadaceae
r2: f__Xanthobacteraceae
s2: f__Sphingomonadaceae
t2: f__bacteriap25
u2: f__Burkholderiaceae
v2: f__Rhodanobacteraceae
w2: f__Rokubacteriales
x2: f__Chthoniobacteraceae
y2: g__Bryobacter
z2: g__Subgroup_2
a3: g__RB41
b3: g__Subgroup_6
c3: g__IMCC26256
d3: g__Acidothermus
e3: g__Arthrobacter
f3: g__MB_A2_108
g3: g__Gaiella
h3: g__67_14
i3: g__AD3
j3: g__KD4_96
k3: g__Gemmatimonas
l3: g__Saccharimonadales
m3: g__Bradyrhizobium
n3: g__Pseudolabrys
o3: g__bacteriap25
p3: g__Burkholderia_Caballeronia_Paraburkholderia
q3: g__Rhodanobacter
r3: g__Rokubacteriales
s3: g__Candidatus_Udaeobacter
The current LDA threshold is 2.61
PPKa PPKb PPKc
a: p__Acidobacteria
b: p__Actinobacteria
c: p__Armatimonadetes
d: p__Bacteroidetes
e: p__Chloroflexi
f: p__FBP
g: p__Planctomycetes
h: p__Proteobacteria
i: c__Acidimicrobiia
j: c__Actinobacteria
k: c__Thermoleophilia
l: c__Chthonomonadetes
m: c__Fimbriimonadia
n: c__Bacteroidia
o: c__Rhodothermia
p: c__Chloroflexia
q: c__FBP
r: c__Planctomycetacia
s: c__Deltaproteobacteria
t: o__Blastocatellales
u: o__Frankiales
v: o__Micrococcales
w: o__Pseudonocardiales
x: o__Streptomycetales
y: o__Solirubrobacterales
z: o__Chthonomonadales
a1: o__Fimbriimonadales
b1: o__Cytophagales
c1: o__Rhodothermales
d1: o__Thermomicrobiales
e1: o__FBP
f1: o__Isosphaerales
g1: o__Caedibacterales
h1: o__Paracaedibacterales
i1: o__Rhodobacterales
j1: o__Tistrellales
k1: o__Myxococcales
l1: o__Betaproteobacteriales
m1: f__Intrasporangiaceae
n1: f__Rhodothermaceae
o1: f__Chloroflexaceae
p1: f__Paracaedibacteraceae
q1: f__Sandaracinaceae
r1: f__Methylophilaceae
s1: f__Blastocatellaceae
t1: f__Frankiaceae
u1: f__Geodermatophilaceae
v1: f__Microbacteriaceae
w1: f__Pseudonocardiaceae
x1: f__Streptomycetaceae
y1: f__Solirubrobacteraceae
z1: f__Chthonomonadaceae
a2: f__Fimbriimonadaceae
b2: f__Hymenobacteraceae
c2: f__Flavobacteriaceae
d2: f__JG30_KF_CM45
e2: f__FBP
f2: f__Acidaminococcaceae
g2: f__Isosphaeraceae
h2: f__Caedibacteraceae
i2: f__Rhodobacteraceae
j2: f__Geminicoccaceae
k2: f__P3OB_42
l2: f__Burkholderiaceae
m2: g__Stenotrophobacter
n2: g__Jatrophihabitans
o2: g__Modestobacter
p2: g__Amnibacterium
q2: g__Curtobacterium
r2: g__Microbacterium
s2: g__Rathayibacter
t2: g__Friedmanniella
u2: g__Actinomycetospora
v2: g__E1B_B3_114
w2: g__Conexibacter
x2: g__Parviterribacter
y2: g__Patulibacter
z2: g__Chthonomonas
a3: g__Fimbriimonadaceae
b3: g__Flaviaesturariibacter
c3: g__Hymenobacter
d3: g__Larkinella
e3: g__Flavobacterium
f3: g__JG30_KF_CM45
g3: g__Crinalium_SAG_22_89
h3: g__FBP
i3: g__Phascolarctobacterium
j3: g__Singulisphaera
k3: g__Acidiphilium
l3: g__Endobacter
m3: g__Caedibacter
n3: g__Paracoccus
o3: g__Rubellimicrobium
p3: g__MN_122_2a
q3: g__Sphingoaurantiacus
r3: g__Candidatus_Alysiosphaera
s3: g__P3OB_42
t3: g__Aquabacterium
u3: g__Massilia
The current LDA threshold is 2.9
RPKa RPKb RPKc
a: p__Chalara
b: p__Rozellomycota
c: p__Wardomyces
d: p__Arthrocatena
e: p__Ascomycota
f: p__Basidiomycota
g: p__Mucoromycota
h: p__Staphylotrichum
i: c__Arthrocatena_tenebrio
j: c__Staphylotrichum_coccosporum
k: c__Dothideomycetes
l: c__Eurotiomycetes
m: c__Lecanoromycetes
n: c__Leotiomycetes
o: c__Pezizomycetes
p: c__Sordariomycetes
q: c__Agaricomycetes
r: c__Geminibasidiomycetes
s: c__Archaeosporomycetes
t: c__Umbelopsidomycetes
u: o__Lecanorales
v: o__Archaeosporales
w: o__Capnodiales
x: o__Pleosporales
y: o__Chaetothyriales
z: o__Eurotiales
a1: o__Helotiales
b1: o__Thelebolales
c1: o__Pezizales
d1: o__Hypocreales
e1: o__Sordariales
f1: o__Agaricales
g1: o__Atheliales
h1: o__Boletales
i1: o__Hymenochaetales
j1: o__Sebacinales
k1: o__Thelephorales
l1: o__Trechisporales
m1: o__Geminibasidiales
n1: o__Cystofilobasidiales
o1: o__Filobasidiales
p1: o__Umbelopsidales
q1: f__Hyaloscyphaceae
r1: f__Clavicipitaceae
s1: f__Pleosporaceae
t1: f__Herpotrichiellaceae
u1: f__Trichomeriaceae
v1: f__Aspergillaceae
w1: f__Trichocomaceae
x1: f__Helotiaceae
y1: f__Myxotrichaceae
z1: f__Vibrisseaceae
a2: f__Pseudeurotiaceae
b2: f__Pezizaceae
c2: f__Hypocreaceae
d2: f__Nectriaceae
e2: f__Chaetomiaceae
f2: f__Atheliaceae
g2: f__Suillaceae
h2: f__Schizoporaceae
i2: f__Sebacinaceae
j2: f__Thelephoraceae
k2: f__Hydnodontaceae
l2: f__Geminibasidiaceae
m2: f__Cystofilobasidiaceae
n2: f__Piskurozymaceae
o2: f__Umbelopsidaceae
p2: g__Subplenodomus
q2: g__Alternaria
r2: g__Cladophialophora
s2: g__Knufia
t2: g__Aspergillus
u2: g__Penicillium
v2: g__Sagenomella
w2: g__Anguillospora
x2: g__Oidiodendron
y2: g__Phialocephala
z2: g__Pseudogymnoascus
a3: g__Delastria
b3: g__Trichoderma
c3: g__Dactylonectria
d3: g__Fusarium
e3: g__Ilyonectria
f3: g__Nectria
g3: g__Thelonectria
h3: g__Chaetomium
i3: g__Humicola
j3: g__Trichocladium
k3: g__Amphinema
l3: g__Athelopsis
m3: g__Suillus
n3: g__Hyphodontia
o3: g__Helvellosebacina
p3: g__Sebacina
q3: g__Tomentella
r3: g__Trechispora
s3: g__Geminibasidium
t3: g__Cystofilobasidium
u3: g__Solicoccozyma
v3: g__Umbelopsis
The current LDA threshold is 2.55
PPKa PPKb PPKc
a: p__Curvibasidium
b: p__Ascomycota
c: p__Neophaeococcomyces
d: p__Phialemoniopsis
e: p__Pseudovirgaria
f: p__Selenophoma
g: p__Symmetrosporaceae
h: c__Neophaeococcomyces_catenatus
i: c__Phialemoniopsis_curvata
j: c__Pseudovirgaria_grisea
k: c__Selenophoma_mahoniae
l: c__Arthoniomycetes
m: c__Dothideomycetes
n: c__Eurotiomycetes
o: c__Leotiomycetes
p: c__Sordariomycetes
q: c__Agaricomycetes
r: c__Cystobasidiomycetes
s: c__Symmetrospora
t: o__Cystofilobasidiales
u: o__Symmetrospora_coprosmae
v: o__Lichenostigmatales
w: o__Capnodiales
x: o__Dothideales
y: o__Pleosporales
z: o__Chaetothyriales
a1: o__Helotiales
b1: o__Thelebolales
c1: o__Glomerellales
d1: o__Hypocreales
e1: o__Agaricales
f1: o__Hymenochaetales
g1: o__Russulales
h1: o__Erythrobasidiales
i1: o__Microstromatales
j1: f__Cyphellophoraceae
k1: f__Sclerotiniaceae
l1: f__Corticiaceae
m1: f__Phaeococcomycetaceae
n1: f__Aplosporellaceae
o1: f__Mycosphaerellaceae
p1: f__Teratosphaeriaceae
q1: f__Aureobasidiaceae
r1: f__Dothideaceae
s1: f__Coniothyriaceae
t1: f__Didymellaceae
u1: f__Leptosphaeriaceae
v1: f__Phaeosphaeriaceae
w1: f__Pleosporaceae
x1: f__Trichomeriaceae
y1: f__Helotiaceae
z1: f__Pseudeurotiaceae
a2: f__Orbiliaceae
b2: f__Glomerellaceae
c2: f__Nectriaceae
d2: f__Ophiocordycipitaceae
e2: f__Sporocadaceae
f2: f__Schizophyllaceae
g2: f__Schizoporaceae
h2: f__Coriolaceae
i2: f__Peniophoraceae
j2: f__Erythrobasidiaceae
k2: f__Microstromataceae
l2: f__Sirobasidiaceae
m2: f__Tremellaceae
n2: g__Phaeococcomyces
o2: g__Aplosporella
p2: g__Septoria
q2: g__Neocatenulostroma
r2: g__Aureobasidium
s2: g__Rhizosphaera
t2: g__Perusta
u2: g__Coniothyrium
v2: g__Ascochyta
w2: g__Didymella
x2: g__Paraconiothyrium
y2: g__Plenodomus
z2: g__Sclerostagonospora
a3: g__Alternaria
b3: g__Stemphylium
c3: g__Tricladium
d3: g__Pseudogymnoascus
e3: g__Orbilia
f3: g__Wilcoxina
g3: g__Colletotrichum
h3: g__Pseudocosmospora
i3: g__Hirsutella
j3: g__Pestalotiopsis
k3: g__Schizophyllum
l3: g__Hyphodontia
m3: g__Trametes
n3: g__Peniophora
o3: g__Occultifur
p3: g__Erythrobasidium
q3: g__Microstroma
r3: g__Sporobolomyces
s3: g__Filobasidium
t3: g__Derxomyces
u3: g__Sirobasidium
v3: g__Cryptococcus

◂**Fig. 6** LEfSe analysis to identify which microbial taxa (from phylum to genus level) were major contributors to the differences in rhizosphere bacterial (**A**), phyllosphere bacterial (**B**), rhizosphere fungal (**C**), and phyllosphere fungal (**D**) community compositions among different samples. PPKa, the phyllosphere of healthy *Pinus koraiensis*; PPKb, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; PPKc, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage; RPKa, the rhizosphere of healthy *P. koraiensis*; RPKb, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; RPKc, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage

microbial groups crowding out the weaker microbial groups or that microbial species unsuited towards living in infected pines disappeared.

Microbial Community Composition Response to Different Treatments

As shown by a growing body of works [14, 75, 90], we also observed that the microbial compositions from rhizosphere and phyllosphere samples formed distinct clusters. Collectively, these studies suggested that although the assemblies of root-associated bacteria and fungi differ substantially from the phyllosphere microbial communities, both represent a subset of the microbe derived from soil communities and enriched in different plant-associated niches [16, 32]. As previous findings indicated that the infection of plant pathogens could affect the host microbial community [52, 76], we also documented that the PWD had a profound impact on the host rhizosphere bacterial and fungal community and phyllosphere fungal community, which was not complete in line with previous work that demonstrated that the community structure of healthy and diseased trees was only significantly different in the roots, and not in the needles and soil [52]. It has become evident that root exudates are the essential factor determining the structure of the rhizosphere microbial community [5, 91]. The occurrence of pine wilt disease can lead to a decreased secretion of soluble sugar, total sugar, and protein in roots [66], which might have caused the observed difference in the microbial community structure in the rhizosphere.

Intriguingly, overall, the bacterial community compositions were similar (in terms of dominant groups) in all samples, different plant compartments at different stages of disease dominated mainly by Proteobacteria, followed by Actinobacteria, and this finding was consistent with several previous studies that displayed that Proteobacteria and Actinobacteria were the dominant groups in rhizosphere bacterial communities [2, 41] and phyllosphere microorganisms [19, 86]. In addition, these groups represent ubiquitous rhizosphere taxa were detected in various stressed environments [92], while the opposite observations from *Pinus massoniana* infected by *B. xylophilus* showed that Acidobacteria was the predominant species in infected soils [69]. Interestingly, due to *B. xylophilus* infection of *P. koraiensis*, the relative abundances of Acidobacteria, Bacteroidetes, and Proteobacteria were significantly higher in diseased pine roots, and the shifts of Proteobacteria have been observed in previous findings [52], which collectively demonstrated that Proteobacteria might be phytopathogens and parasites in plant tissues and cause a variety of diseases [44]. The root and leaf metabolism of diseased trees was weakened relative to the healthy roots and leaves, resulting in a decreased ability of the root and leaf to adapt to the environment condition and it being easily colonized by microbes. Other investigations indicated that Proteobacteria prefer to grow under nutrient-rich conditions [24], which might explain the high content of Proteobacteria in the diseased roots and leaves. The rhizospheric microbial abundance of *Bryobacter*, *RB41*, and *Bradyrhizobium* was richer in diseased pines. Our findings were similar to the results in rhizosphere bacterial studies on *P. thunbergii* where bacteria in the genus *Bradyrhizobium* were more abundant in soil of wilted trees than in soil of non-infected trees [52].

The abundances of the genus *Massilia*, *Sphingoaurantiacus*, *Acidiphilium*, *Acetobacteraceae*, *Singulisphaera*, *Phascolarctobacterium*, and *Hymenobacter* in diseased needles were significantly higher than those in healthy needles, suggesting an association of particular microbial abundances with the infection of *B. xylophilus* in *P. koraiensis*. What's more, the research from Ma et al. [52] demonstrated that *Massilia* was obviously higher in diseased pines, which supported our results to some extent. The genus *Massilia* belongs to the family Oxalobacteraceae of the class Betaproteobacteria in the phylum Proteobacteria [1]. Members of this genus are characterized as Gram-negative, aerobic, non-spore-forming bacteria [99]. Some *Massilia* can produce cell lysis enzymes that promote tissue lysis [55]. This may be the reason for the presence of *Massilia* in a high abundance in diseased needles.

In our study, Ascomycota and Basidiomycota were the dominant fungal phyla with phyllosphere and rhizosphere samples, and this result was in agreement with previous research [40]. Similar results were obtained in Taxus rhizosphere communities [35] and in tropical grasslands [49]. The majorrhizosphere fungal genera in healthy *P. koraiensis* were *Penicillium* and *Trichoderma*, in agreement with a study from Zhang et al. [97]. Interestingly, *Trichoderma* is an important genus in biocontrol of nematodes because some species produce metabolites harmful to nematodes [93]. The enriched phyllosphere fungal groups in PPKc were *Phaeosphaeriaceae*, *Wilcoxina*, *Pseudocosmospora*, and so on. *Phaeosphaeriaceae* was commonly associated with plants as pathogens, though some are also saprotrophs and parasites on powdery mildews [98]. Thus, it can be seen that plant-associated microbes could influence plant health and fitness [94], resistance to pathogens [4], and ecosystem services.

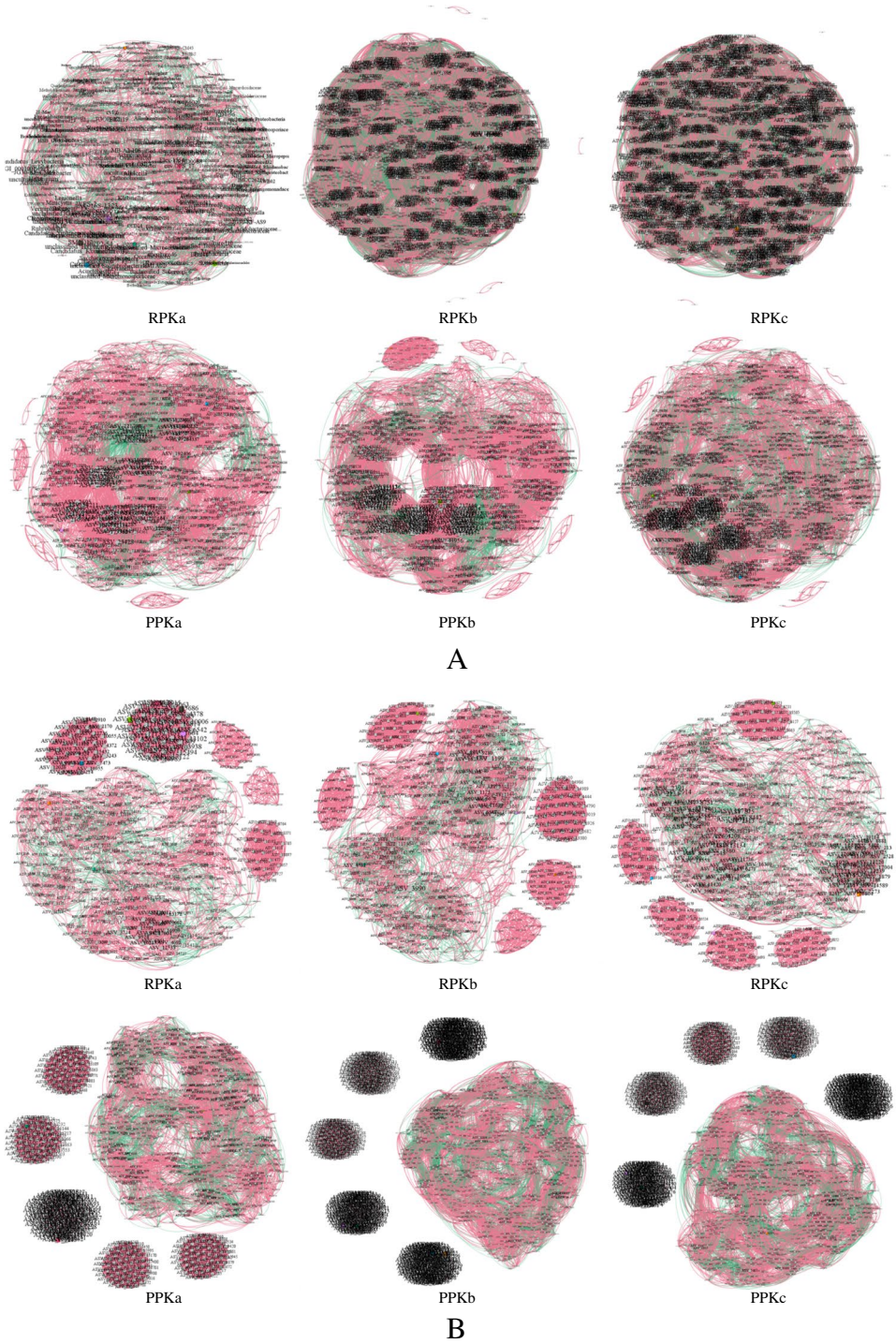


Fig. 7 Network interactions of bacterial (**A**) and fungal (**B**) OTUs (OTUs with the abundance more than 5) from phyllosphere and rhizosphere. Each node represents an OTU, and colors of the nodes indicate different phyla. The OTUs were separated into different modules, shown as circles, by the greedy modularity optimization method. PPKa, the phyllosphere of healthy *Pinus koraiensis*; PPKb, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; PPKc, the phyllosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage; RPKa, the rhizosphere of healthy *P. koraiensis*; RPKb, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the early stage; RPKc, the rhizosphere of *P. koraiensis* naturally infected by *Bursaphelenchus xylophilus* at the last stage

Shifts of co-Occurrence Association Network Response to Different Treatments

In our study, the co-occurrence network of phyllosphere and rhizosphere microbial community demonstrated dynamical relationships between healthy *P. koraiensis* and the infection of *B. xylophilus* in *P. koraiensis*, which could provide momentous details of microbial community assembly and represent interactions among different populations that regulate ecological processes [25]. Total nodes of phyllosphere and rhizosphere microbial community association network increased after *B. xylophilus* infection, indicating that the populations of the ecological network increased after infection (Fig. 7; Table S1), resulting in the microbial diversity to increase in some degree. The edges of phyllosphere and rhizosphere microbial community association network

existed higher in *P. koraiensis* infected by *B. xylophilus* than healthy *P. koraiensis*, which depicted changes among nodes, reflecting their responses to environmental perturbations [70]. Furthermore, the role of microbial co-occurrence networks is important in revealing the interactions (such as through parasitism, competition, and mutualism) that exist among different species [21, 100]. In our study, except for phyllosphere fungi, positive links of phyllosphere bacteria, rhizosphere bacteria, and fungi decrease with the infection of PWD, and at the last stage, positive links existed the lowest (Fig. 7; Table S1), demonstrating that most of the microbial taxa tended to be in competition rather than mutualism.

Conclusions

Overall, an increase in diversity with more severe symptomatic stage was visible. What's more, the microbial compositions from rhizosphere samples and phyllosphere samples formed distinct clusters. Rhizosphere bacterial and fungal community, and phyllosphere fungal community from PKa, PKb, and PKc formed three distinct clusters, which clearly separated along the PCoA1. These findings manifested that the phyllosphere and rhizosphere microbial community changed potentially caused by *B. xylophilus* infection of *P. koraiensis*. Furthermore, LEfSe analysis demonstrated that variations of some microbial abundances were associated with the infection of *B. xylophilus* in *P. koraiensis*, including *Bradyrhizobium* (rhizosphere bacteria), *Massilia* (phyllosphere bacteria), and *Phaeosphaeriaceae* (phyllosphere fungi). With the infection of PWD, most of the bacterial taxa tended to be co-excluding rather than co-occurring. Together, our results explored PWD could increase the phyllosphere and rhizosphere microbial community diversity and microbial community composition differed as the disease progressed, and these changes would correlate with microbial ability to suppress plant pathogen. This study expanded our knowledge of the ecology of plant-microbe interactions as well as the structure and assembly of microbial communities of healthy *P. koraiensis* and the infection of *B. xylophilus* in *P. koraiensis*, which lay the foundation for studies that aim at improving plant growth by altering the plant microbiome.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-021-01850-4.

Author Contribution Jiaojiao Deng and Wenxu Zhu conceived and designed the experiments. Jiaojiao Deng and Wangming Zhou performed the experiments and analyzed the data. Jiaojiao Deng and Li Zhou prepared the figures and tables and wrote the original draft preparation. Wenxu Zhu, Li Zhou, and Dapao Yu authored or reviewed drafts of the paper.

Funding This research was financially supported by the Mission of Chinese Academy of Sciences (E0381601).

Data Availability The high-throughput sequencing raw data of phyllosphere and rhizosphere microbes were uploaded in the NCBI database with the SRA accession numbers PRJNA689361 and PRJNA689392.

Code Availability R scripts are available in the supplementary materials.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

References

1. Altankhuu K, Kim J (2017) Massilia solisilvae sp. nov. Massilia terrae sp. nov. and Massilia agilis sp. nov. isolated from forest soil in South Korea by using a newly developed culture method. Int J Syst Evol Micr 67(8):3026. https://doi.org/10.1099/ijsem.0.002076
2. Alvarez-López V, Prieto-Fernández Á, Becerra-Castro C, Monterroso C, Kidd P (2016) Rhizobacterial communities associated with the flora of three serpentine outcrops of the Iberian Peninsula. Plant Soil 403(1–2):233–252. https://doi.org/10.1007/s11104-015-2632-0
3. Alves M, Pereira A, Vicente CSL, Matos PN, Henriques J, Lopes H, Nascimento FX, Mota M, Correia ACM, Henriques I (2018) The role of bacteria in pine wilt disease: insights from microbiome analysis. FEMS Microbiol. Ecol. 94(7):fiy077. https://doi.org/10.1093/femsec/fiy077
4. Awasthi A, Singh M, Soni SK, Singh R, Kalra A (2014) Biodiversity acts as insurance of productivity of bacterial communities under abiotic perturbations. ISME J 8(12):2445–2452. https://doi.org/10.1038/ismej.2014.91
5. Badri DV, Chaparro JM, Zhang RF, Shen QR, Vivanco JM (2013) Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. J. Biol. Chem. 288(7):4502–4512. https://doi.org/10.1074/jbc.M112.433300
6. Bais HP, Loyola-Vargas VM, Flores HE, Vivanco JM (2001) Root-specific metabolism: the biology and biochemistry of underground organs. Vitro Cell Dev-Pl 37(6):730–741. https://doi.org/10.1007/s11627-001-0122-y
7. Bal AS (2003) Isolation of endophytic bacteria from lodgepole pine (*Pinus contorta* var. latifolia (Dougl.) Engelm.) and western red cedar (*Thuja plicata* Donn.) and determination of their nitrogen fixing ability
8. Bao LJ, Cai WY, Cao JX, Zhang XF, Liu JH, Chen H, Wei YS, Zhuang XL, Zhuang GQ, Bai ZH (2020) Microbial community overlap between the phyllosphere and rhizosphere of three plants from Yongxing Island, South China Sea. Microbiology Open 9(7):e1048. https://doi.org/10.1002/mbo3.1048
9. Beule L, Grüning M, Karlovsky P, L-M-Arnold A (2017) Changes of scots pine phyllosphere and soil fungal communities during outbreaks of defoliating insects. Forests 8(9):316. https://doi.org/10.3390/f8090316
10. Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P (2013) Structure and functions of the bacterial

microbiota of plants. Annu. Rev. Plant Biol. 64(1):807–838. https://doi.org/10.1146/annurev-arplant-050312-120106
11. Carrell A, Frank AC (2014) *Pinus flexilis* and *Picea engelmannii* share a simple and consistent needle endophyte microbiota with a potential role in nitrogen fixation. Front. Microbiol. 5:333. https://doi.org/10.3389/fmicb.2014.00333
12. Cavicchioli R, Ripple WJ, Timmis K, Azam F, Bakken LR, Baylis M, Behrenfeld MJ, Boetius A, Boyd PW, Classen AT, Crowther TW, Danovaro R, Foreman CM, Huisman J, Hutchins DA, Jansson JK, Karl DM, Koskella B, Welch DBM et al (2019) Scientists' warning to humanity: microorganisms and climate change. Nat Rev Microbiol 17(9):569–586. https://doi.org/10.1038/s41579-019-0222-5
13. Chen HJ, Hao DJ, Wei ZQ, Wang LJ, Lin T (2020a) Bacterial communities associated with the pine wilt disease insect vector Monochamus alternatus (Coleoptera: Cerambycidae) during the larvae and pupae stages. Insects 11(6):376. https://doi.org/10.3390/insects11060376
14. Chen PL, Zhao ML, Tang F, Hu YM, Peng XJ, Shen S (2020b) The effect of plant compartments on the Broussonetia papyrifera-associated fungal and bacterial communities. Appl Microbiol Biot 104(8):3627–3641. https://doi.org/10.1007/s00253-020-10466-6
15. Chialva M, Salvioli A, Daghino S, Ghignone S, Bagnaresi P, Chiapello M, Novero M, Spadaro D, Perotto S, Bonfante P (2018) Native soils with their microbiotas elicit a state of alert in tomato plants. New Phytol. 220(4):1296–1308. https://doi.org/10.1111/nph.15014
16. Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross SM, Clingenpeel S, Woyke T, North GB, Visel A, Partida-Martinez LP, Tringe SG (2016) Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. New Phytol. 209(2):798–811. https://doi.org/10.1111/nph.13697
17. David AS, Quintana-Ascencio PF, Menges ES, Thapa-Magar K, Searcy C, Afkhami ME (2019) Soil microbiomes underlie population persistence of an endangered plant species. Am. Nat. 194(4):488–494. https://doi.org/10.1086/704684
18. De Zelicourt A, Ai-Yousif M, Hirt H (2013) Rhizosphere microbes as essential partners for plant stress tolerance. Mol. Plant 6(2):242–245. https://doi.org/10.1093/mp/sst028
19. Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R, Mering C, Vorholt JA (2009) Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. P Natl Acad Sci USA 106(38):16428–16433. https://doi.org/10.1073/pnas.0905240106
20. Deng JJ, Bai XJ, Zhou YB, Zhu WX, Yin Y (2020) Variations of soil microbial communities accompanied by different vegetation restoration in an open-cut iron mining area. Sci. Total Environ. 704:135243. https://doi.org/10.1016/j.scitotenv.2019.135243
21. Deng Y, Jiang YH, Yang YF, He ZL, Luo F, Zhou JZ (2012) Molecular ecological network analyses. BMC Bioinformatics 13(1):113. https://doi.org/10.1186/1471-2105-13-113
22. Dong CJ, Wang LL, Li Q, Shang QM (2019) Bacterial communities in the rhizosphere, phyllosphere and endosphere of tomato plants. PLoS One 14(11):e0223847. https://doi.org/10.1371/journal.pone.0223847
23. Douanla-Meli C, Langer E, Talontsi Mouafo F (2013) Fungal endophyte diversity and community patterns in healthy and yellowing leaves of Citrus Limon. Fungal Ecol. 6(3):212–222. https://doi.org/10.1016/j.funeco.2013.01.004
24. Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology 88(6):1354–1364. https://doi.org/10.1890/05-1839
25. Fuhrman JA (2009) Microbial community structure and its functional implications. Nature 459(7244):193–199. https://doi.org/10.1038/nature08058
26. Fukuda K (1997) Physiological process of the symptom development and resistance mechanism in pine wilt disease. J Forest Res 2(3):171–181. https://doi.org/10.1007/BF02348216
27. Futai K (2008) Pine wilt in Japan: from first incidence to the present. Pine Wilt Disease:5–12. https://doi.org/10.1007/978-4-431-75655-2_2
28. Genitsaris S, Stefanidou N, Leontidou K, Matsi T, Karamanoli K, Ifigeneia M (2020) Bacterial communities in the rhizosphere and phyllosphere of halophytes and drought-tolerant plants in Mediterranean ecosystems. Microorganisms 8(11):1708. https://doi.org/10.3390/microorganisms8111708
29. Ghelardini L, Pepori A, Luchi N, Capretti P, Santini A (2016) Drivers of emerging fungal diseases of forest trees. Forest Ecol Manag 381:235–246. https://doi.org/10.1016/j.foreco.2016.09.032
30. Goldford JE, Lu N, Bajić D, Estrela S, Tikhonov M, Sánchez-Gorostiaga A, Segrè D, Mehta P, Sanchez A (2018) Emergent simplicity in microbial community assembly. Science 361(6401):469–474. https://doi.org/10.1101/205831
31. Guo QQ, Guo DS, Zhao BG, Xu J, Li RG (2007) Two cyclic dipeptides from Pseudomonas fluorescens gcm5-1a carried by the pine wood nematode and their toxicities to Japanese black pine suspension cells and seedlings in vitro. J. Nematol. 39(3):243–247
32. Hamonts K, Trivedi P, Garg A, Janitz C, Grinyer J, Holford P, Botha FC, Anderson IC, Singh B (2018) Field study reveals core plant microbiota and relative importance of their drivers. Environ. Microbiol. 20(1):124–140. https://doi.org/10.1111/1462-2920.14031
33. Han HC, Chung YJ, Shin SC (2008) First report of pine wilt disease on *Pinus koraiensis* in Korea. Plant Dis. 92(8):1251–1251. https://doi.org/10.1094/PDIS-92-8-1251A
34. Han ZM, Hong YD, Zhao BG (2003) A study on pathogenicity of bacteria carried by pine wood nematodes. J. Phytopathol. 151(11–12):683–689. https://doi.org/10.1046/j.1439-0434.2003.00790.x
35. Hao DC, Song SM, Mu J, Hu WL, Xiao PG (2016) Unearthing microbial diversity of Taxus rhizosphere via miseq high-throughput amplicon sequencing and isolate characterization. Sci. Rep. 6:22006. https://doi.org/10.1038/srep22006
36. Hardoim PR, Van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol R 79(3):293–320. https://doi.org/10.1128/MMBR.00050-14
37. Hayat S, Faraz A, Faizan M (2017) Root exudates: composition and impact on plant-microbe interaction. Biofilms in Plant and Soil Health:179–193. https://doi.org/10.1002/9781119246329.ch10
38. Izumi H, Anderson IC, Killham K, Moore ERB (2008) Diversity of predominant endophytic bacteria in European deciduous and coniferous trees. Can. J. Microbiol. 54(3):173–179. https://doi.org/10.1139/w07-134
39. Jia T, Guo TY, Cao MW, Chai BF (2018) Effects of heavy metals on phyllosphere and rhizosphere microbial community of Bothriochloa ischaemum. Appl. Sci. 8(9):1419. https://doi.org/10.3390/app8091419
40. Jia T, Yao Y, Guo T, Wang R, Chai B (2020) Effects of plant and soil characteristics on phyllosphere and rhizosphere fungal communities during plant development in a copper tailings dam.

Front. Microbiol. 11:556002. https://doi.org/10.3389/fmicb.2020.556002
41. Jorquera MA, Maruyama F, Ogram AV, Navarrete OU, Lagos LM, Inostroza NG, Acuña JJ, Rilling JI, de La Luz MM (2016) Rhizobacterial community structures associated with native plants grown in Chilean extreme environments. Microb. Ecol. 72(3):633–646. https://doi.org/10.1007/s00248-016-0813-x
42. Kawai M, Shoda-kagaya E, Maehara T, Zhou ZH, Lian CL, Iwata R, Yamane A, Hogetsu T (2006) Genetic structure of pine sawyer Monochamus alternatus (Coleoptera: Cerambycidae) populations in Northeast Asia: consequences of the spread of pine wilt disease. Environ. Entomol. 35(2):569–579. https://doi.org/10.1007/s11814-009-0145-3
43. Kembel SW, Mueller RC (2014) Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities. Botany 92(92):159. https://doi.org/10.1139/cjb-2013-0194
44. Kersters K, Vos PD, Gillis M, Swings J, Stackebrandt E (2006) Introduction to the proteobacteria. The prokaryotes:3–37. https://doi.org/10.1007/0-387-30745-1_1
45. Kobayashi DY, Crouch JA (2009) Bacterial/fungal interactions: from pathogens to mutualistic endosymbionts. Annu. Rev. Phytopathol. 47(1):63–82. https://doi.org/10.1146/annurev-phyto-080508-081729
46. Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P et al (2013) Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22(21):5271–5277. https://doi.org/10.1111/mec.12481
47. Layeghifard M, Hwang DM, Guttman DS (2017) Disentangling interactions in the microbiome: a network perspective. Trends Microbiol. 25(3):217–228. https://doi.org/10.1016/j.tim.2016.11.008
48. Li X, Zhang HH, Sun ML, Xu N, Sun GY, Zhao MC (2020) Land use change from upland to paddy field in Mollisols drives soil aggregation and associated microbial communities. Appl. Soil Ecol. 146:103351. https://doi.org/10.1016/j.apsoil.2019.09.001
49. Lienhard P, Terrat S, Prevost-Boure NC, Nowak V, Régnier T, Sayphoummie S, Panyasiri K, Tivet F, Mathieu O, Lévêque J, Maron PA, Ranjard L (2014) Pyrosequencing evidences the impact of cropping on soil bacterial and fungal diversity in Laos tropical grassland. Agron. Sustain. Dev. 34(2):525–533. https://doi.org/10.1007/s13593-013-0162-9
50. Liu KC, Ben A, Han ZM, Guo Y, Cao DX (2019) Interspecific hybridization between *Bursaphelenchus xylophilus* and *Bursaphelenchus mucronatus*. J Forestry Res 30(2):699–707
51. Lu T, Ke MJ, Lavoie M, Jin YJ, Fan XJ, Zhang ZY, Fu ZW, Sun LW, Gillings MR, Peñuelas J, Qian HF, Zhu YG (2018) Rhizosphere microorganisms can influence the timing of plant flowering. Microbiome 6(1):231. https://doi.org/10.1186/s40168-018-0615-0
52. Ma Y, Qu ZL, Liu B, Tan JJ, Asiegbu FO, Sun H (2020) Bacterial community structure of *Pinus thunbergii* naturally infected by the nematode *Bursaphelenchus xylophilus*. Microorganisms 8(2):307. https://doi.org/10.3390/microorganisms8020307
53. Maehara N (2008) Reduction of *Bursaphelenchus xylophilus* (Nematoda: Parasitaphelenchidae) population by inoculating Trichoderma spp. into pine wilt-killed trees. Biol. Control 44(1):61–66. https://doi.org/10.1016/j.biocontrol.2007.09.005
54. Mannaa M, Han G, Jeon HW, Kim J, Kim N, Park AR, Kim JC, Seo YS (2020) Influence of resistance-inducing chemical elicitors against pine wilt disease on the rhizosphere microbiome. Microorganisms 8(6):884. https://doi.org/10.3390/microorganisms8060884
55. Maya O, Yitzhak H, Dror M, Alexander VB (2012) Ecology of root colonizing Massilia (Oxalobacteraceae). PLoS One 7(7):e40117. https://doi.org/10.1371/journal.pone.0040117
56. Metz MR, Frangioso KM, Wickland AC, Meentemeyer RK, Rizzo DM, Metz C (2012) An emergent disease causes directional changes in forest species composition in coastal California. Ecosphere 3(10):1–23. https://doi.org/10.1890/ES12-00107.1
57. Millberg H, Boberg J, Stenlid J (2015) Changes in fungal community of scots pine (*Pinus sylvestris*) needles along a latitudinal gradient in Sweden. Fungal Ecol.:126–139. https://doi.org/10.1016/j.funeco.2015.05.012
58. Naylor D, Sadler NC, Bhattacharjee A, Graham E, Anderton CR, McClure R, Lipton M, Hofmockel KS, Jansson JK (2020) Soil microbiomes under climate change and implications for carbon cycling. Ann Rev Environ Resour 45:29–59. https://doi.org/10.1146/annurev-environ-012320-082720
59. Pan L, Li YX, Liu ZK, Meng FL, Chen J, Zhang XY (2019) Isolation and identification of pine wood nematode in *Pinus koraiensis* in Fengcheng, Liaoning Province. Forest Pest and Disease 38(1):1–4. https://doi.org/10.19688/j.cnki.issn1671-0886.20180021
60. Preston D, Mischler J, Townsend A, Johnson P (2016) Disease ecology meets ecosystem science. Ecosystems 19(4):737–748. https://doi.org/10.1007/s10021-016-9965-2
61. Proena DN, Grass G, Morais PV (2017) Understanding pine wilt disease: roles of the pine endophytic bacteria and of the bacteria carried by the disease-causing pinewood nematode. Microbiology Open 6(2):e00415. https://doi.org/10.1002/mbo3.415
62. Proença DN, Francisco R, Kublik S, SchöLer A, Vestergaard G, Schloter M, Morais PV (2017) The microbiome of endophytic, wood colonizing bacteria from pine trees as affected by pine wilt disease. Sci. Rep. 7(1):4205. https://doi.org/10.1038/s41598-017-04141-6
63. Qiu XW, Wu XQ, Huang L, Ye JR (2016) Influence of Bxpel1 gene silencing by dsRNA interference on the development and pathogenicity of the pine wood nematode, *Bursaphelenchus xylophilus*. Int. J. Mol. Sci. 17(1):125. https://doi.org/10.3390/ijms17010125
64. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41(D1):D590–D596. https://doi.org/10.1093/nar/gks1219
65. Ren G, Zhu CW, Alam MS, Tokida T, Sakai H, Nakamura H, Usui Y, Zhu JG, Hasegawa T, Jia ZJ (2015) Response of soil, leaf endosphere and phyllosphere bacterial communities to elevated CO_2 and soil temperature in a rice paddy. Plant Soil 392(1–2):27–44. https://doi.org/10.1007/s11104-015-2503-8
66. Reva V, Fonseca L, Lousada JL, Abrantes I, Viegas DX (2012) Impact of the pinewood nematode, Bursaphelenchus xylophilus, on gross calorific value and chemical composition of *Pinus pinaster* woody biomass. Eur J Forest Res 131(4):1025–1033. https://doi.org/10.1007/s10342-011-0574-5
67. Rodrigues JM (2008) Pine wilt disease: a worldwide threat to forest ecosystems. Springer Netherlands. https://doi.org/10.1007/978-1-4020-8455-3_1
68. Rodriguez PA, Rothballer M, Chowdhury SP, Nussbaumer T, Falter-Braun P (2019) Systems biology of plant-microbiome interactions. Mol. Plant 12(6):804–821. https://doi.org/10.1016/j.molp.2019.05.006
69. Shi C, Wang C, Xu X, Huang B, Wu L, Yang D (2015) Comparison of bacterial communities in soil between nematode-infected and nematode-uninfected *Pinus massoniana* pinewood forest. Appl. Soil Ecol. 85:11–20. https://doi.org/10.1016/j.apsoil.2014.08.008

70. Shi S, Nuccio EE, Shi ZJ, He Z, Zhou J, Firestone MK (2016) The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. Ecol. Lett. 19(8):926–936. https://doi.org/10.1111/ele.12630
71. Spaargaren OC, Deckers J (1998) The world reference base for soil resources. In: Schulte A, Ruhiyat D (eds) Soils of Tropical Forest Ecosystems. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-03649-5_2
72. Stone BWG, Weingarten EA, Jackson CR (2018) The role of the phyllosphere microbiome in plant health and function. American Cancer Society
73. Sun ML, Li T, Li DM, Zhao YL, Gao FM, Sun LF, Li X (2021) Conversion of land use from upland to paddy field changes soil bacterial community structure in Mollisols of Northeast China. Microb. Ecol. 81(4):1018–1028. https://doi.org/10.1007/s00248-020-01632-4
74. Tan JJ, Xiang HQ, Feng ZX (2008) A preliminary study on isolation, identification and pathogenicity of the bacterium accompanying *Bursaphelenchus xylophilus*. China Forestry Science and Technology 22:23–26
75. Tardif S, Yergeau É, Tremblay J, Legendre P, Whyte LG, Greer CW (2016) The willow microbiome is influenced by soil petroleum-hydrocarbon concentration with plant compartment-specific effects. Frontiers Microbiol 7:1363. https://doi.org/10.3389/fmicb.2016.01363
76. Tian BY, Cao Y, Zhang KQ (2015) Metagenomic insights into communities, functions of endophytes, and their associates with infection by root-knot nematode, Meloidogyne incognita, in tomato roots. Sci. Rep. 5:17087. https://doi.org/10.1038/srep17087
77. Toju H, Peay K, Yamamichi M, Narisawa K, Hiruma K, Naito K, Fukuda S, Ushio M, Nakaoka S, Onoda Y, Yoshida K, Schlaeppi K, Bai Y, Sugiura R, Ichihashi Y, Minamisawa K, Kiers T (2018) Core microbiomes for sustainable agroecosystems. Nat Plants 4:247–257. https://doi.org/10.1038/s41477-018-0139-4
78. Tóth Á (2011) Bursaphelenchus xylophilus, the pinewood nematode: its significance and a historical review. Acta Biologica Szegediensis 55(2):213–217
79. Vacher C, Hampe A, Porté AJ, Sauer U, Compant S, Morris CE (2016) The phyllosphere: microbial jungle at the plant-climate interface. Annu Rev Ecol Evol S 47:1–24. https://doi.org/10.1146/ANNUREV-ECOLSYS-121415-032238
80. Van Der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11(3):296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x
81. Vandenkoornhuyse P, Quaiser A, Duhamel M, Van AL, Dufresne A (2015) The importance of the microbiome of the plant holobiont. New Phytol. 206(4):1196–1206. https://doi.org/10.1111/nph.13312
82. Venturi V, Keel C (2016) Signaling in the rhizosphere. Trends Plant Sci. 187. https://doi.org/10.1016/j.tplants.2016.01.005
83. Vicente C, Espada M, Vieira P, Mota M (2012) Pine wilt disease: a threat to European forestry. Eur. J. Plant Pathol. 133(1):89–99. https://doi.org/10.1007/s10658-012-9979-3
84. Vicente CSL, Ikuyo Y, Mota M, Hasegawa K (2013) Pinewood nematode-associated bacteria contribute to oxidative stress resistance of Bursaphelenchus xylophilus. BMC Microbiol. 13:299. https://doi.org/10.1186/1471-2180-13-299
85. Vives-Peris V, Molina L, Segura A, Gómez-Cadenas A, Pérez-Clemente RM (2018) Root exudates from citrus plants subjected to abiotic stress conditions have a positive effect on rhizobacteria. J. Plant Physiol. 228:208–217. https://doi.org/10.1016/j.jplph.2018.06.003
86. Vokou D, Genitsaris S, Karamanoli K, Vareli K, Sainis I (2019) Metagenomic characterization reveals pronounced seasonality in the diversity and structure of the phyllosphere bacterial community in a Mediterranean ecosystem. Microorganisms 7(11):518–536. https://doi.org/10.3390/microorganisms7110518
87. Vorholt JA (2012) Microbial life in the phyllosphere. Nat Rev Microbiol 10(12):828–840. https://doi.org/10.1038/nrmicro2910
88. Wagner MR, Lundberg DS, Rio TGD, Tringe SG, Dangl JL, Mitchell-Olds T (2016) Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. Nat. Commun. 7:12151. https://doi.org/10.1038/ncomms12151
89. Wang Z, Zhang Y, Wang C, Wang Y, Sung C (2017) Esteya vermicola controls the pinewood nematode, *Bursaphelenchus xylophilus*, in pine seedlings. J Nematol 49(1):86-91. https://doi.org/10.21307/jofnem-2017-048
90. Xiang Q, Chen QL, Zhu D, Yang XY, Qiao M, Hu HW, Zhu YG (2020) Microbial functional traits in phyllosphere are more sensitive to anthropogenic disturbance than in soil. Environ. Pollut. 265(PtA):114954. https://doi.org/10.1016/j.envpol.2020.114954
91. Xu C, Wu X (2016) Physiological and proteomic analysis of mycorrhizal *Pinus massoniana* inoculated with Lactarius insulsus under drought stress. Russ J Plant Physl 63(5):709–717. https://doi.org/10.1134/S1021443716040178
92. Yadav AN, Verma P, Kumar S, Kumar V, Kumar M, Sugitha T, Singh BP, Saxena A, Dhaliwal HS (2018) Actinobacteria from rhizosphere: molecular diversity, distributions, and potential biotechnological applications. Actinobacteria: Diversity and Biotechnological Applications 13–41. https://doi.org/10.1016/B978-0-444-63994-3.00002-3
93. Yang Z, Yu Z, Lei L, Xia Z, Shao L, Zhang K, Li G (2012) Nematicidal effect of volatiles produced by Trichoderma sp. J. Asia Pac. Entomol. 15(4):647–650. https://doi.org/10.1016/j.aspen.2012.08.002
94. Zamioudis C, Pieterse CMJ (2012) Modulation of host immunity by beneficial microbes. Mol Plant-Microbe In 25(2):139–150. https://doi.org/10.1094/MPMI-06-11-0179
95. Zamora P, Rodríguez V, Renedo F, Sanz AV, Domínguez JC, Pérez-Escolar G, Miranda J, Álvarez B, González-Casas A, Mayor E, Dueñas M, Miravalles A, Navas A, Robertson L, Martín AB (2015) First report of *Bursaphelenchus xylophilus* causing pine wilt disease on *Pinus radiata* in Spain. Plant Dis. 99(10):1449–1449. https://doi.org/10.1094/PDIS-03-15-0252-PDN
96. Zhang N, Wang D, Liu Y, Li S, Shen Q, Zhang R (2014) Effects of different plant root exudates and their organic acid components on chemotaxis, biofilm formation and colonization by beneficial rhizosphere-associated bacterial strains. Plant Soil 374(1–2):689–700. https://doi.org/10.1007/s11104-013-1915-6
97. Zhang W, Wang X, Li YX, Liu Z, Li D, Wen X, Feng Y, Zhang XY (2020) Pinewood nematode alters the endophytic and rhizospheric microbial communities of *Pinus massoniana*. Microb. Ecol. 81(3):807–817. https://doi.org/10.1007/s00248-020-01619-1
98. Zhang Y, Crous PW, Schoch CL, Hyde KD (2012) Pleosporales. Fungal Divers. 53(1):1–221. https://doi.org/10.1007/s13225-011-0117-x
99. Zhao X, Li X, Qi N, Gan M, Pan Y, Han T, Hu X (2017) Massilia neuiana sp. nov. isolated from wet soil. Int J Syst Evol Micr 67(12):4943–4947. https://doi.org/10.1099/ijsem.0.002333
100. Zhou J, Deng Y, Luo F, He Z, Yang Y (2011) Phylogenetic molecular ecological network of soil microbial communities in response to elevated CO_2. Mbio 2(4):e00122–e00111. https://doi.org/10.1128/mBio.00122-11
101. Zhou LF, Chen FM, Xie LY, Pan HY, Ye JR (2017) Genetic diversity of pine-parasitic nematodes *Bursaphelenchus*

xylophilus and *Bursaphelenchus mucronatus* in China. Forest Pathol 47(4):e12334. https://doi.org/10.1111/efp.12334

102. Zhou Q, Zhang X, He R, Wang S, Jiao C, Huang R, He X, Zeng J, Zhao D (2019) The composition and assembly of bacterial communities across the rhizosphere and phyllosphere compartments of *Phragmites australis*. Diversity-Basel 11(6):98. https://doi.org/10.3390/d11060098
103. Zhu LH, Ye J, Negi S, Xu XL, Wang ZL, Ji JY (2012) Pathogenicity of aseptic *Bursaphelenchus xylophilus*. PLoS One 7(5):e38095. https://doi.org/10.1371/journal.pone.0038095